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REPORT ON THE SHRIMP VIRUS PEER REVIEW AND RISK ASSESSMENT WORKSHOP

Developing A Qualitative Ecological Risk Assessment

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CONTENTS

APPENDIX B. **Peer Review Experts and Breakout Discussion Assignments**

APPENDIX C. **Premeeting Comments Prepared by Workshop Experts**

APPENDIX D. **Workshop Agenda**

APPENDIX E. **Presentation Materials on the Risk Assessment Process Developed by the Aquatic Nuisance Species Task Force**

APPENDIX F. **Summary Materials Presented by Workshop and Breakout Group Chairs**

APPENDIX G. **Report to the Aquatic Nuisance Species Task Force: Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process**

APPENDIX H. **Observers' Comments and List of Observers**

LIST OF FIGURES

EXECUTIVE SUMMARY

This report highlights issues and conclusions from the shrimp virus peer review workshop sponsored by the U.S. Environmental Protection Agency (EPA) in cooperation with the Joint Subcommittee on Aquaculture (JSA), held January 7–8, 1998, in Arlington, Virginia. The goals of the workshop were to:

- Complete a qualitative assessment of the risks associated with shrimp viruses, following the general risk assessment process developed by the Aquatic Nuisance Species Task Force.
- Evaluate the need for a future, more comprehensive risk assessment.
- Identify critical risk-relevant research needs.

The workshop focused on the scientific and technical aspects of the likelihood that nonindigenous viruses will become established in wild shrimp populations in the Gulf of Mexico and southeastern Atlantic coastal regions and on the potential ecological consequences of establishment. The workshop included 22 experts with varied backgrounds, including shrimp biology, toxicology, virology, marine ecology, ecological risk assessment, and shrimp aquaculture and processing. Prior to the workshop, participants received several background documents (ERG, 1997; JSA, 1997; RAM, 1996 [Appendix G]) and prepared written premeeting comments that all participants reviewed (Appendix C). At the workshop, participants were divided into three groups, each of which was charged with evaluating the risks associated with one of the following categories of viral pathways:

- Aquaculture
- Shrimp processing
- Other potential sources

The risk that shrimp viruses pose to shrimp aquaculture operations was not considered as part of the scope of the workshop due to the limited time available; however, workshop participants believed that the risks to shrimp in aquaculture should be given special attention as part of a subsequent technical or management workshop.

The qualitative risk assessment was conducted using the modified Aquatic Nuisance Species Task Force risk assessment approach (RAM, 1996; Appendix G). In developing the qualitative risk assessment, participants considered the following:

- Likelihood of viruses being present in the pathway
- Ability of the viruses to survive transit in the pathway
- Colonization potential of the viruses in native shrimp
- Spread potential of the virus within native shrimp populations
- Consequences of establishment

In general, workshop participants agreed that viruses could be associated with pathways leading to coastal environments and that they could survive in these pathways. Participants concluded that there is potential for viruses to colonize native shrimp in localized areas, such as an estuary or embayment, near the point of entry into the marine system. Some participants also noted that repeated viral introductions to an area will increase the risk of colonization.

Participants had widely divergent views on the potential for viruses to spread beyond the initial local area of colonization. This divergence largely reflects the high uncertainty associated with this aspect of exposure. Participants considered the potential for localized colonization and subsequent spread to be a critical aspect of evaluating the potential establishment of viruses in native shrimp.

Workshop participants discussed the impact that virus establishment could have on local shrimp populations (e.g., within an individual estuary). They determined that initial kill rates might be high but that the population would be likely to recover rapidly due to reintroduction of shrimp from other locales or compensatory increases in reproduction. Workshop participants concluded that the risk from viral introductions to the entire population of native shrimp along the southeastern Atlantic coast and within the Gulf of Mexico is relatively low, although there is a high degree of uncertainty associated with this evaluation.

The ability of workshop participants to address broader ecological risks in a comprehensive manner was limited by the time and information available. However, some participants thought that the issue of broader ecological risks is important and merits further consideration.

Workshop participants identified areas where further research and information would improve the assessment of risks and could help evaluate current conditions. They also identified actions for reducing uncertainty that should be given the highest priority, including:

- Improved diagnostic methods
- Surveys of wild shrimp populations for presence of the four nonindigenous viruses and for genetic composition
- Experiments to reduce uncertainties surrounding virus transmission and virulence
- Field epidemiological studies

Participants identified other areas where additional research is needed to improve the ability to estimate risks to wild shrimp populations, including:

- Viral persistence
- Compensatory mechanisms
- Monitoring of imported shrimp
- Development of suitable population models

- Targeted surveys of nonpenaeid species to determine if they are susceptible to or carriers of nonindigenous viruses

Workshop participants believed that, given the existing knowledge base, it is currently not feasible to conduct a more comprehensive, quantitative assessment of the risks associated with shrimp viruses. Participants generally agreed that, at present, qualitative evaluations could be made, but there is a great deal of uncertainty associated with this type of process. Participants determined that there is a need to continue efforts to gather available data on shrimp virus effects and a need to conduct a systematic research effort that could be used to reduce the uncertainty of any subsequent risk assessments.

Workshop participants identified the following areas of concern where additional efforts should be focused:

- Management implications of shrimp viruses
- Risks of shrimp viruses to aquaculture operations
- Risks of shrimp viruses to nonpenaeid species

1. INTRODUCTION

This report highlights issues and conclusions from a shrimp virus peer review workshop sponsored by the U.S. Environmental Protection Agency (EPA) in cooperation with the Joint Subcommittee on Aquaculture (JSA), held January 7–8, 1998, in Arlington, Virginia. The goals of the workshop were to:

- Complete a qualitative assessment of the risks associated with shrimp viruses, following the general risk assessment process developed by the Aquatic Nuisance Species Task Force
- Evaluate the need for a future, more comprehensive risk assessment
- Identify critical risk-relevant research needs

The workshop focused on the scientific and technical aspects of the likelihood that nonindigenous viruses will become established in wild shrimp populations in the Gulf of Mexico and southeastern Atlantic coastal regions and on the potential consequences of such establishment.

This section provides an overview of the recently published JSA report that formed the basis for the workshop, a description of the workshop process, and a discussion of the qualitative risk assessment approach used at the workshop. Section 2 of the report summarizes discussions held during the workshop on several aspects of the qualitative risk assessment process, and it contains a risk characterization developed by the workshop chair and breakout group chairs following the workshop's conclusion. Section 3 discusses actions for reducing uncertainty that were identified by participants during the workshop. The reports of each breakout group are contained in Appendix A.

1.1 JSA REPORT OVERVIEW

Dr. Kay Austin of EPA's National Center for Environmental Assessment, and a member of the JSA Shrimp Virus Work Group, gave an initial presentation that discussed the Work Group's efforts to date and events leading to the workshop. She provided an overview of the purpose, scope, and findings of the Work Group's report, entitled "An Evaluation of Potential Shrimp Virus Impacts on Cultured Shrimp and on Wild Shrimp Populations in the Gulf of Mexico and Southeastern U.S. Atlantic Coastal Waters" (JSA, 1997; JSA Report). Highlights of her presentation follow.

New, highly virulent viruses have been documented in foreign shrimp aquaculture. Consumer demand for shrimp continues to grow and, to meet this demand, the United States has greatly increased shrimp importation from areas of the world where shrimp viruses are known to be endemic. Recent events have prompted calls for investigation into the actual risks to U.S. domestic resources. These events have included catastrophic viral outbreaks in shrimp aquaculture both in the United States and abroad, recent appearances of these organisms in shrimp in commercial retail stocks, and new information on the susceptibility of shrimp and other crustaceans to these organisms. While some of these viruses have severe and lethal effects in crowded aquaculture conditions, they are not known to pose threats to human health.

The U.S. shrimp industry (harvesting and processing alone) is valued at $3 billion per year. Imported shrimp account for over 80 percent of the market. In 1995, imports exceeded domestic production by a ratio of four to one, amounting to 720 million pounds (in tails). The largest share of these imports comes from Latin America and Asia—areas of the world where shrimp viruses are endemic. Domestic aquaculture operations, in contrast, account for a much smaller portion of the U.S. market, ranging from 2 million pounds in 1991 to 4 million pounds in 1994.

The JSA, which is under the auspices of the President's Office of Science and Technology Policy, formed the interagency Shrimp Virus Work Group in March 1996 to assess the risks associated with these emerging viral pathogens. Four federal agencies are represented in the Work Group: the National Marine Fisheries Service (NMFS), the U.S. Environmental Protection Agency (EPA), the U.S. Fish and Wildlife Service (USFWS), and the U.S. Animal and Plant Health Inspection Service (APHIS). JSA charged the Work Group with developing a federal interagency strategy to address the shrimp virus issue and to identify relevant research on viral stressors, their potential mode of transmission, and their potential for introduction to U.S. shrimp resources.

The Work Group recognized that the shrimp virus problem presents some unique issues in risk assessment. They determined that the problem is a complex one that moves beyond the traditional single-chemical, single-species assessment process. The shrimp virus problem involves potentially nonindigenous viral stressors and has great potential to significantly impact the U.S. shrimp industry and other ecological components of coastal systems.

During its initial evaluation of the problem, the Work Group decided to base its approach on EPA's ecological risk assessment guidelines, which were published in draft form in 1996 (U.S. EPA, 1996). Because the Work Group determined that not enough information was available to complete an actual risk assessment, it followed a problem formulation approach that enabled the Work Group to summarize risk-relevant information available prior to January 1997 and to identify data gaps and critical research needs.

During its problem formulation activities, the Work Group developed a proposed management goal and identified potential viral sources, potential viral and other environmental stressors, and potential ecological effects. The Work Group also reached consensus on assessment endpoints and developed a conceptual model (Figure 1) that illustrates the linkages between human activities, viral stressors, and assessment endpoints of concern. The Work Group's report (JSA 1997; JSA Report) was published in June 1997.

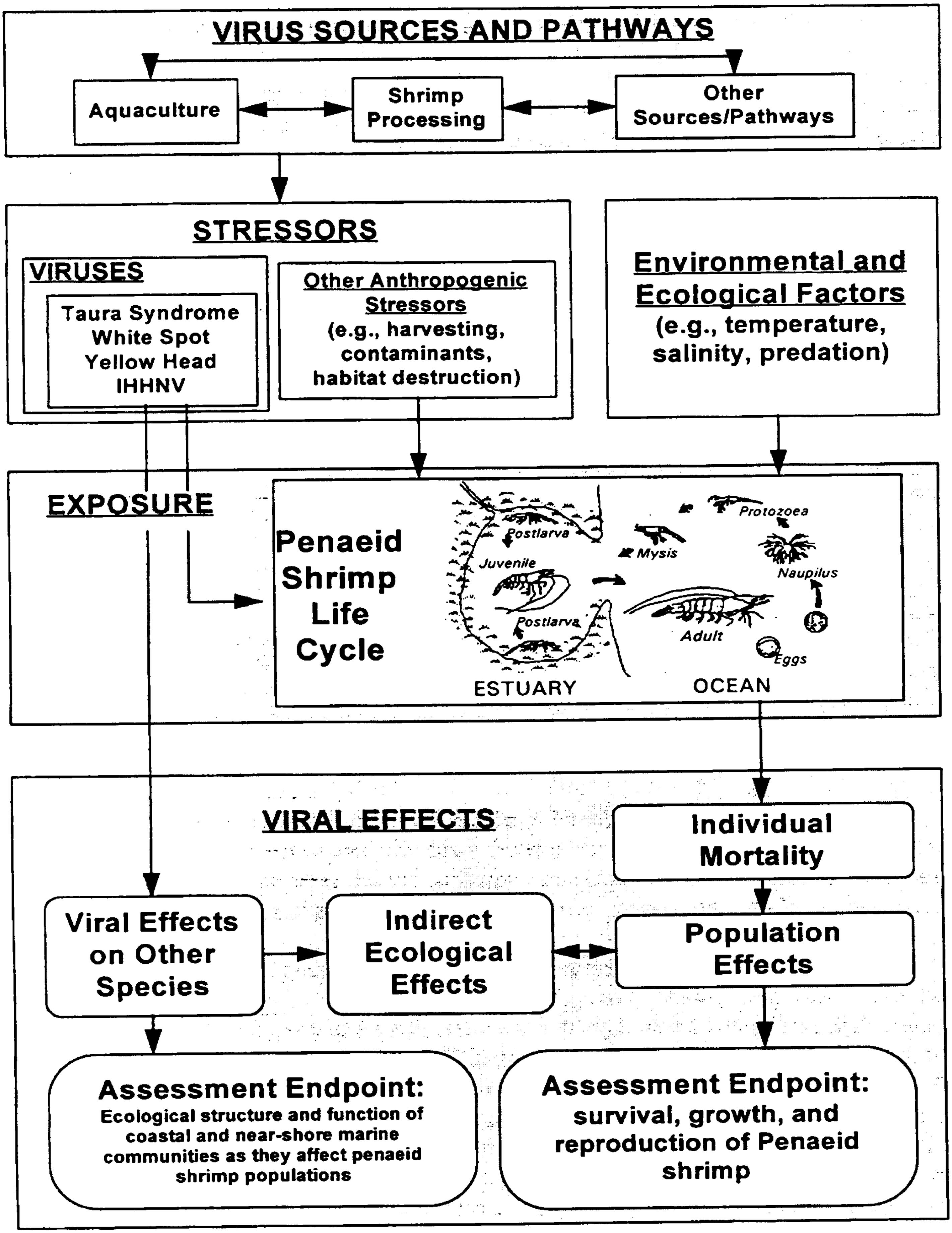

Figure 1. Proposed Shrimp Virus Conceptual Model.

Significant findings of the report include:

- Viral disease has been associated with severe declines in wild shrimp harvests in the Gulf of California. Populations of the blue shrimp, *Penaeus stylirostris*, and other less dominant species plummeted coincident with the observed occurrence of IHHNV disease in wild shrimp populations in the Gulf of California. The Work Group found that this is the best piece of epidemiological information suggesting a link between introduced viruses and declines in wild shrimp populations. There remains considerable debate, however, regarding the validity of this association of disease and effects.

- Nonindigenous shrimp viruses have not been documented in U.S. shrimp populations; until recently, detection efforts have been minimal. Sampling techniques may have been inadequate, and the correct technology may not have been available to adequately detect the viruses.

- Numerous viral disease outbreaks have occurred in U.S. shrimp aquaculture since 1994, and frozen shrimp in commerce have been found to be contaminated with these viruses. Laboratory studies show that all life stages of shrimp are potentially at risk from at least one of the four viruses covered by this report.

- Harvesting practices in foreign aquaculture could put U.S. domestic populations at risk. The Work Group learned that when an outbreak occurs in some foreign aquaculture operations, the affected crop is often harvested immediately and exported to avoid severe crop and monetary losses.

- Shrimp may be contaminated from a number of possible sources. The Work Group identified aquaculture and shrimp processing as two potentially important sources that may affect wild shrimp populations. The Work Group also considered a number of other possible sources, such as live and frozen bait shrimp, ballast water, and natural spread by mechanisms such as hurricanes, floods, or animals. Research and display facilities may also be a source of exposure to wild populations.

- Species other than shrimp may be at risk from these viruses. Viral disease could result in alterations to ecosystem structure or function, potentially affecting a wide range of endpoints, such as predator-prey relationships, competition, and nutrient cycling. Many other economically and ecologically important organisms that occupy coastal areas feed on juvenile shrimp, and impacts to these organisms could be serious if the wild shrimp populations on which they feed decline. Other organisms may be susceptible to disease themselves or serve as carriers of these viruses.

During July 1997, JSA and EPA sponsored public meetings in Charleston, South Carolina; Mobile, Alabama; Brownsville, Texas; and Thibodaux, Louisiana to gather stakeholder input on the shrimp virus issue and the JSA report. Stakeholders included individuals from the wild shrimp fishery industry, the shrimp aquaculture industry, the shrimp processing industry, environmental organizations, regulatory and resource management agencies, and the general public. The minutes of these stakeholder meetings were published in October 1997 (ERG, 1997).

1.2 PEER REVIEW WORKSHOP PROCESS

At the beginning of the workshop, the workshop chair, Dr. Charles Menzie of Menzie-Cura & Associates, reviewed the agenda (Appendix D), explained the workshop's format, and reviewed the workshop's goals, which were to:

- Complete a qualitative assessment of the risks associated with shrimp viruses, following the general risk assessment process developed by the Aquatic Nuisance Species Task Force
- Evaluate the need for a future, more comprehensive risk assessment
- Identify critical risk-relevant research needs

Dr. Menzie explained that the workshop report would be used to provide input to a proposed workshop to identify potential risk management options. The proposed workshop, sponsored by JSA and NMFS, is tentatively scheduled for July 1998.

Prior to the workshop, ERG provided each expert with the JSA report (JSA, 1997), the minutes of the stakeholder meetings about the JSA report (ERG, 1997), and a copy of a qualitative risk assessment process for nonindigenous organisms (RAM, 1996; Appendix G). ERG asked panel members (Appendix B) to review the material and prepare written comments to address questions on the following topics:

- Management goals, assessment endpoints, and the conceptual model
- Viral stressors and factors regulating shrimp populations
- Viral pathways and sources
- Stressor effects
- Comprehensive risk assessment and research needs

The charge to experts and experts' premeeting comments are contained in Appendix C. Overheads prepared by the chairs that summarize the premeeting comments are contained in Appendix F. Peer review experts were divided into three breakout groups, each of which was charged with evaluating the risks associated with one of three viral pathways (aquaculture, shrimp processing, and other potential sources).

Three experts in ecological risk assessment were selected as breakout group leaders: Dr. Wayne Munns (EPA Office of Research and Development), who facilitated discussions on aquaculture; Dr. John Gentile (University of Miami), who facilitated discussions on shrimp processing; and Dr. Anne Fairbrother (Ecological Planning and Toxicology, Inc.), who facilitated discussions on “other potential sources.” (See Appendix B for breakout group assignments.) After the workshop, Dr. Menzie prepared the qualitative risk assessment (Section 2), Dr. Munns prepared the report of the Aquaculture Breakout Group (Appendix A-1), Dr. Gentile prepared the report of the Shrimp Processing Breakout Group (Appendix A-2), and Dr. Fairbrother prepared the report of the “Other Pathways” Breakout Group (Appendix A-3). Workshop participants had a chance to review and comment on the report prior to preparation of the final document.

1.3 QUALITATIVE RISK ASSESSMENT METHODOLOGY

Mr. Richard Orr, of the U.S. Department of Agriculture, Animal, and Plant Health Inspection Services (USDA-APHIS), provided participants with an overview of the qualitative risk assessment methodology to be used at the workshop. The process is based on the Aquatic Nuisance Species Task Force (ANSTF) risk assessment approach (RAM, 1996, Appendix G), which provides a qualitative assessment of the probability and consequences of establishment of a nonindigenous species in a new environment. Mr. Orr noted that the methodology may be used as a subjective evaluation, or it may be quantified to the extent possible depending on the needs of the analysis. He reviewed an assessment on black carp to illustrate the application of this process to a nonindigenous species. Both documents were provided to workshop experts as background information prior to the workshop.

Mr. Orr explained that the risk assessment model is divided into two major components: the “probability of establishment” and the “consequences of establishment” (see Figure 2, which contains the risk assessment model from the Report to the Aquatic Nuisance Species Task Force). These components of the model are further divided into basic elements that serve to focus scientific, technical, and other relevant information for the assessment. Mr. Orr discussed how the following elements could be used to estimate the probability of establishment of viral pathogens in wild shrimp populations:

- Probability of the nonindigenous organism being on, with, or in the pathway
- Probability of the organism surviving in transit
- Probability of the organism successfully colonizing and maintaining a population where introduced
- Probability of the organism spreading beyond the colonized area

The following elements are used in the ANSTF approach to evaluate the consequence of establishment of a nonindigenous species (see page 22, Appendix G):

- Economic impact
- Environmental impact
- Impact from social and/or political influences

For the purposes of the Shrimp Virus Peer Review Workshop, only environmental impacts were evaluated. Economic and perceived impacts of establishment may be considered at the proposed workshop on risk management options, scheduled for July 1998.

Mr. Orr stressed that it is critical for the qualitative risk assessment to capture and communicate the uncertainty that surrounds the available information about shrimp viruses.

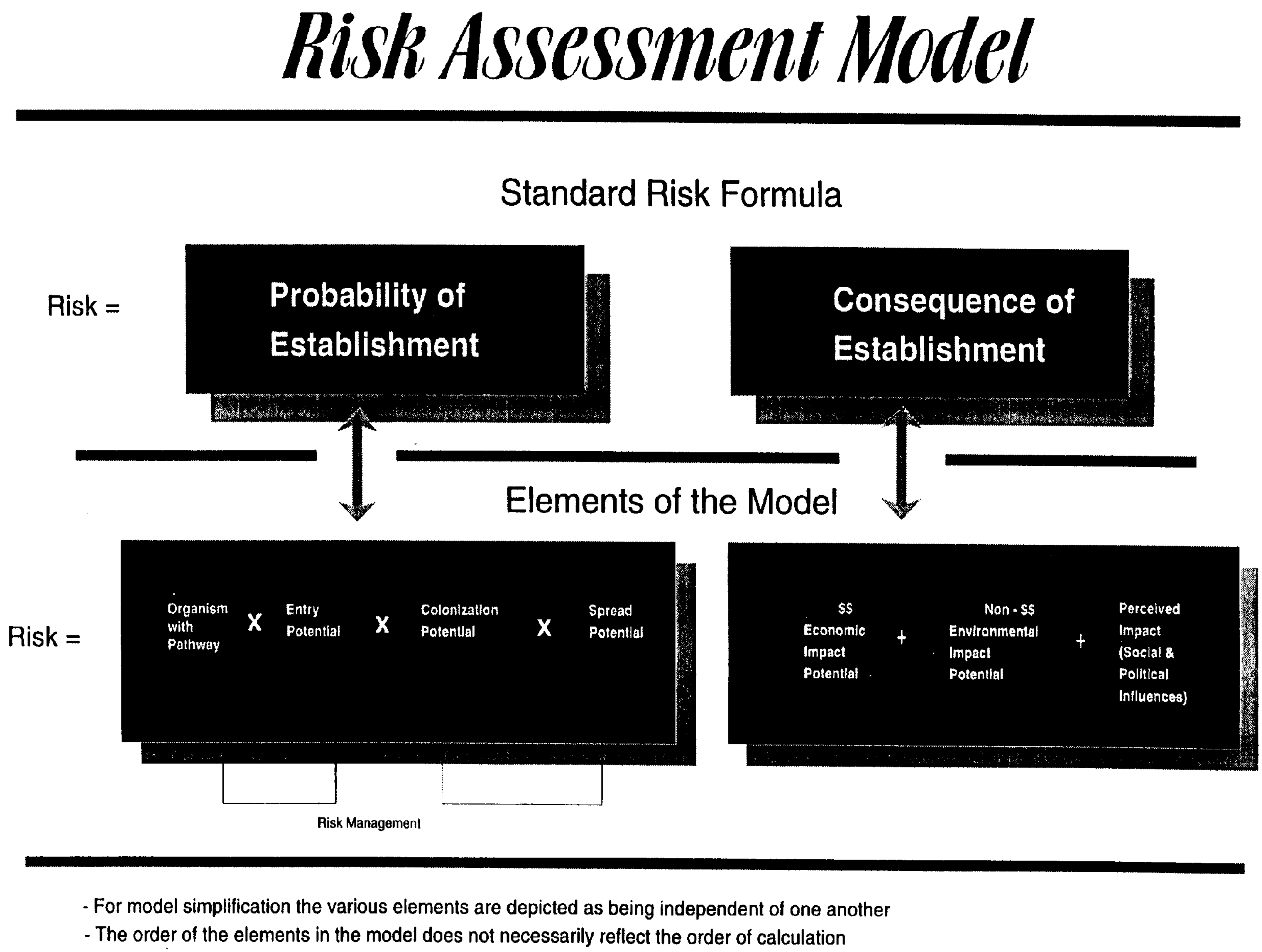

Figure 2. Risk Assessment Model from the Report to the Aquatic Nuisance Species Task Force (RAM, 19

2. QUALITATIVE RISK ASSESSMENT

2.1 THE RISK ASSESSMENT PROCESS

Workshop participants began the risk assessment process by reviewing the management goal and assessment endpoints presented in the JSA report (JSA, 1997). Participants evaluated the risks associated with aquaculture, shrimp processing, or other potential sources. In the breakout groups, participants considered the ecological risks associated with each identified viral pathway. The evaluation of each pathway was conducted independently. It is important to note that participants did not attempt to rank the relative risk of the three identified sources.

Each breakout group evaluated both the potential for establishment of the viruses via the identified pathways and the potential ecological consequences of establishment. The breakout groups considered the four following elements of the potential for establishment of viruses via the identified pathways:

- Association of nonindigenous viruses with the pathway
- Entry of nonindigenous viruses into coastal waters via the pathway (including survival)
- Colonization/infection of shrimp at the local level
- Spread of nonindigenous viruses to the shrimp populations at large

To determine the probability of establishment of nonindigenous viruses, the breakout groups rated each of these elements low, medium, or high. The consequences of establishment were similarly rated. During their deliberations, the breakout groups were asked to identify the level of uncertainty (ranging from very uncertain to very certain) associated with each of the elements described previously.

Using the method set forth in the ANSTF report (Appendix G), workshop participants estimated the overall risk by compiling the risks associated with the individual elements of the process (i.e., [1] the four elements of the probability of establishment and [2] the consequence of establishment). The probability of establishment is determined by the lowest ranking of any of the four elements. For example, if elements under the probability of establishment had rankings of high, high, low, and medium, the overall probability of establishment would be considered low, because establishment is determined by the lowest likelihood of any one element. Rankings for the probability of establishment and the consequence of establishment may be combined into an overall level of risk as follows:

If the Overall Probability of Establishment Is:	And the Consequence of Establishment Is:	Then the Overall Risk Ranking Is:
High	High	High
Medium	High	High
Low	High	Medium
High	Medium	High
Medium	Medium	Medium
Low	Medium	Medium
High	Low	Medium
Medium	Low	Medium
Low	Low	Low

These rankings, which are based on judgment, should not be considered separately from the discussion and rationale provided by the workshop participants. As noted in the ANSTF report (RAM, 1996), "the strength of the Review Process is not in the element-rating but in the detailed biological and other relevant information statements that motivate them."

After evaluating the probability of establishment for their respective pathways and the consequences of establishment at the local and regional (e.g., Gulf of Mexico) population levels, the three breakout groups presented their findings in a plenary session. Following the conclusion of the expert workshop, the breakout group chairs and the workshop chair met to develop a risk characterization for the assessment.

2.2 QUALITATIVE RISK ASSESSMENT RESULTS

This section summarizes discussions held during the workshop on several aspects of the risk assessment process:

- Management goals and assessment endpoints that frame the assessment (Section 2.2.1)
- The probability of establishment of shrimp viruses (Section 2.2.2)
- The consequences of establishment (Section 2.2.3)
- A characterization of the risks resulting from a combination of the probability and consequences of establishment (Section 2.2.4)

The reports of the three breakout groups are contained in Appendix A.

2.2.1 Management Goals and Assessment Endpoints

Workshop participants were asked to evaluate the completeness and adequacy of both the management goal and the assessment endpoints identified in the JSA report (JSA, 1997). In the ecological risk assessment process, the management goal is intended to reflect the management context of the assessment, while the assessment endpoints are explicit expressions of the environmental values to be protected, which serve as the focal points for an assessment.

The management goal identified in the JSA report is to:

- Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. coastal waters while minimizing possible impacts on shrimp importation, processing, and aquaculture operations.

A number of participants thought that the management goal should be broadened to include risks to aquaculture operations. Participants concurred that these risks are important but, because of the limited time available for workshop discussions, they agreed that risks to aquaculture operations would not be considered during the workshop. Participants recommended instead that risks to shrimp in aquaculture operations and management of those risks be the subject of a separate workshop.

The JSA report identifies two assessment endpoints:

- Survival, growth, and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters
- Ecological structure and function of coastal and near-shore marine communities as they affect wild penaeid shrimp populations

Workshop participants elected to focus their efforts on the first assessment endpoint (direct effects to wild shrimp populations) for the following reasons:

- Risks to wild shrimp populations are of primary concern
- Information on secondary effects is even more limited than information on direct effects on shrimp
- There was limited time available at the workshop for evaluating all possible direct and indirect effects.

Participants recognized the potential for direct effects on organisms other than penaeid shrimp and the potential for indirect effects; however, these effects were not discussed in detail during the workshop. They are, however, a potential concern for resource managers.

2.2.2 Probability of Establishment

This section summarizes breakout group discussions concerning the elements of the probability of establishment (association with pathway, entry potential, colonization [infection] potential, and spread potential).

Workshop participants recognized that differences among the four viruses could result in variations in the risk rankings associated with the elements comprising the probability of establishment. For example, if one virus were to survive longer than another virus in the marine environment, it could affect the entry potential ranking. However, the breakout groups decided to consider the viruses as a single group but to identify any unique differences that might alter risk rankings. A summary of the characteristics of the four viruses is contained in Table 2-1.

2.2.2.1 Association with the Pathway

Breakout groups concluded with moderate to high certainty that there is a high likelihood that viruses are present in the aquaculture pathway, shrimp processing pathway, and some of the other potential pathways.

<u>Aquaculture</u>. The occurrence of nonindigenous viruses in U.S. aquaculture operations is well documented. As summarized in the JSA report, TSV has been identified in disease outbreaks in Hawaii, Texas, and South Carolina (Lightner, 1996a, 1996b). IHHNV was first identified in Hawaii (Lightner et al., 1983a, 1983b) and was subsequently observed in farms in South Carolina, Texas, and Florida (Fulks & Main, 1992). WSSV and YHV also have been documented at a shrimp farm in Texas (Lightner, 1996a, 1996b). WSSV and YHV are considered to be of Asian origin; TSV and IHHNV are thought to have originated in Latin America. Workshop participants noted that the origins of these viruses are not always traceable to their ultimate sources, but it was suggested that their introduction to the United States may have resulted from the importation of infected shrimp from other regions of the world (e.g., Latin America and Asia).

<u>Shrimp Processing</u>. Shrimp viruses can be brought into the United States with imported shrimp that are subsequently processed or used for other purposes (e.g., feed, bait shrimp, and retail sale). Of the shrimp processed in the United States, 80 percent of the total crop is foreign and 20 percent is domestic in origin. Pathogenic viruses have been identified in imported shrimp sold in this country.

<u>Other Pathways</u>. Other "primary" pathways described in the JSA report and considered by workshop participants include ballast water, bait shrimp, animal vectors, and shrimp feed. There appears to be no data on the occurrence of shrimp viruses in ballast water (or any of its components). Nonetheless, it is known that many organisms are discharged routinely with ballast water (including species of mysid shrimp, some of which have colonized bays and estuaries with devastating effects). There is therefore a high probability that ballast water could

Table 2-1. Virus persistence, virulence, and infectivity

	IHHNV	TSV	YHV	WSSV
Persistence (1 = most, 4 = least)	**3.5**	**3.5**	**1.5**	**1.5**
Virulence to Gulf Species (1 = most, 4 = least)	**1**	**2**	**3**	**4**
	Relative Infectivity			
Penaeus setiferus				
Larvae	—	—	**ND**	**ND**
Post-larvae	—	++	—	++
Juvenile	+	+	++	++
Adult	**ND**	+	**ND**	**ND**
Penaeus duorarum				
Larvae	—	—	**ND**	**ND**
Post-larvae	—	—	—	++
Juvenile	+	+	++	+
Adult	**ND**	**ND**	**ND**	**ND**
Penaeus aztecus				
Larvae	—	—	**ND**	**ND**
Post-larvae	—	+	—	++
Juvenile	+	+	++	+
Adult	**ND**	**ND**	**ND**	

INFECTIVITY

ND = No data
+ = Infectious
++ = Mortality
— = Tried but negative

contain shrimp viruses, whether free living, attached to particulate matter, or in dead or infected shrimp.

Anglers use shrimp as bait when fishing in estuaries for fish that eat shrimp. They purchase bait from bait shops or they use shrimp sold in grocery stores for human consumption. Bait shrimp generally are smaller than those sold for human consumption and are often considered substandard. They may originate from aquaculture facilities that have harvested their shrimp prior to full growout because of a viral outbreak. Some participants thought that Latin American and Asian producers may freeze these small shrimp and ship them to the United States for sale as bait, while the larger, uninfected shrimp will be sold at premium prices for human consumption. Therefore, there is a high probability that some imported bait shrimp may contain viruses.

Both live and frozen shrimp may be sold as bait. However, only native species of aquaculture shrimp may be harvested and sold as live bait. Some states (e.g., South Carolina) allow the use of nonnative farm shrimp as frozen bait. Native shrimp used in aquaculture are known to sometimes carry indigenous viruses (such as BP, another baculovirus) but, to date, there is no evidence that they carry nonindigenous viruses. Furthermore, any of these shrimp that are harvested early due to perceived disease problems are likely to be sold as frozen bait rather than as fresh bait. Therefore, there is a low probability that live shrimp used for bait will carry nonindigenous viruses.

Shrimp feed is made from soy protein, fish protein (including anchovies and menhaden), shrimp heads, and other types of shrimp and crustaceans (e.g., *Artemia*). Because the heads and other body parts of infected shrimp can carry a high concentration of viruses, workshop participants believed that there is a high probability that the shrimp parts used as an ingredient in shrimp feed may be contaminated with the viruses. Although viruses may be associated with this pathway, workshop participants concluded that they are likely to be destroyed during processing of the shrimp feed (see Section 2.2.2.2).

Animal vectors such as gulls and freshwater and marine invertebrates were considered as another possible source for viral entry. For example, gulls and other scavengers, such as raccoons, are often seen feeding on dead shrimp and other organic matter associated with aquaculture facilities that have undergone viral outbreaks. Workshop participants believed there was a high probability of viral association with this pathway.

Workshop participants considered a number of other pathways to have a low probability for viral association; therefore, these pathways—natural spread of the viruses, research and display facilities, human sewage, fishing vessels, hobby and ornamental displays, live seafood distribution, other crustacean aquaculture, and incidental introductions—were not discussed in detail at the workshop.

2.2.2.2 Entry Potential

Entry potential includes the probability of viruses surviving in transit and the probability of their transport to coastal waters. Each breakout group recognized that the entry potential of nonindigenous viruses depends on the pathway of arrival. For example, the survival and entry characteristics of viruses found in shrimp processing effluents may be quite different from those found in ship ballast waters. In addition, the breakout groups recognized that entry potential depends on location. For example, viruses associated with shrimp that are raised, processed, or disposed of in locations far inland are less likely to reach coastal waters than are viruses that are associated with shrimp that are raised, processed, or disposed of along the coast. Workshop participants evaluated subpathways within each of the major pathway categories (aquaculture, shrimp processing, and other source pathways) and described entry potentials for viruses as ranging from low to high. Participants found the level of certainty associated with these evaluations to be quite variable.

<u>Aquaculture</u>. The Aquaculture Breakout Group considered the six subpathways from aquaculture to wild shrimp stocks identified in the conceptual model contained in the JSA report. Many breakout group members believed that the escapement subpathway (including both accidental and intentional releases, as well as "escape" via transport of shrimp tissue by the predatory activities of other animals) was the most likely route of release of viruses to the environment and that viruses were likely to survive when transported via this pathway. (As discussed in the following, however, some breakout group members believed that the sediment and effluent pathways, which the group tabled because of a lack of crucial data, may also be important.)

The Aquaculture Breakout Group noted that the entry potential via escapement (and other pathways) is likely to be related to the conditions in the pond (i.e., the presence and degree of infection by the viruses), the life stage of the shrimp (e.g., postlarvae may be more likely than adult shrimp to escape by passing through engineering controls), and the design of pond control systems. They concluded with relatively high certainty that the probability of surviving in transit would be high if conditions are favorable but assigned a low probability of survival if they are not.

The Aquaculture Breakout Group had considerable discussion about the ability of viruses to survive in pond effluents and sediments. There is suggestive evidence about this potential pathway. TSV has been documented in water, but not specifically in effluent waters. A workshop observer (R. Laramore) communicated results of an experiment that suggest that caged shrimp exposed in infected ponds developed disease. (Shrimp developed disease when exposed within 1 to 2 days to experimentally inoculated water, but they did not develop disease when exposed on days 3 to 5 following inoculation [R. Laramore]). In 1995, HSF, Ltd., and the Arroyo Aquaculture Association conducted several trials in which cages were floated within a shrimp growout pond that had experienced a TSV epidemic and with pond water in tanks. The cages were suspended above the pond bottom and stocked with juvenile *P. vannamei.*

No TSV was detected in shrimp exposed for 30 days under these conditions (F. Jaenike, personal communication). These results suggest that TSV may be transmitted during the acute but not the chronic stages of the disease. Other data suggest that IHHNV can survive in water in an infective state for at least 24 days (Glover et al., 1995). One participant noted that viruses can spread quickly from pond to pond on aquaculture facilities, but it is not known how this transmission occurs. Based on this information, the aquaculture breakout group estimated that there is a medium potential that effluents released from infected farm ponds are a viable pathway for exposure to native populations; however, the breakout group was very uncertain about this estimate.

Shrimp Processing. The Shrimp Processing Breakout Group identified two subpathways for which there is a moderate to high potential for viruses to enter coastal areas: untreated effluents from shrimp processing facilities and solid wastes from disposal facilities near coastal areas that receive waste from shrimp processing facilities. The breakout group concluded with high certainty that there is a low potential for viable shrimp viruses to survive in effluents that are treated and disinfected at municipal facilities and, therefore, there is a low potential for entry of viable shrimp viruses to coastal areas from this pathway.

The Shrimp Processing Breakout Group estimated that approximately 50 percent of shrimp processing liquid effluent is untreated and that virus-contaminated discharges may therefore be released regularly into the environment. The breakout group was very certain that the probability of the organism surviving in transit, and therefore entering the environment through this pathway, is high.

Because of the uncertainties associated with the amounts of material reaching landfills, the types of vectors, and the threshold amount of virus required to infect the wild and aquaculture populations, the Shrimp Processing Breakout Group found it difficult to assess the probability of establishment of shrimp viruses from solid waste disposal facilities. Most breakout group members generally agreed that the shells, and particularly the heads, of foreign farmed and wild shrimp are highly likely to contain viruses. Considering these factors, breakout group participants concluded that these viruses are likely to persist for some time in landfill settings. Land crabs and seagulls are thought to be primary vectors for moving viruses from the landfills to estuarine waters. When these animals consume virus-contaminated materials, some of the viruses (TSV and IHHNV) can pass through their digestive systems in an infective state. It was noted that TSV remains infective following gut passage in gulls, waterboatman, and other insects. It is not known whether the concentrations and frequency of virus introduction from these vectors is sufficient to infect wild and aquaculture shrimp populations. The breakout group was reasonably certain that there is a moderate probability of entry potential from coastal landfills to estuaries.

Other Pathways. The "Other Pathways" Breakout Group found that the entry potential of viruses in ballast water and bait shrimp is high. The group determined that while it is not likely that the freezing process used for bait shrimp will significantly reduce the virulence and infectivity of the virus, the effects of freezing may be virus specific. For shrimp feed, participants concluded that the probability of survival in transit depends on whether or not the feed meal is heat treated to temperatures sufficient to inactivate all viruses. It is thought that some of the viruses (e.g., TSV) may survive and maintain infectivity, even when heated to temperatures greater than 100 °C. While most of the fish meal produced in the United States is subjected to temperatures that appear to be sufficient to kill the viruses, breakout group members were unable to provide published data that would confirm this supposition. Moreover, several participants believed that other countries do not always heat-treat their meals, which would increase the potential for viable viruses to be present in the feed. The "Other Pathways" Breakout Group concluded that the transit survival probability is low for heat-treated feed and high for untreated feed. In contrast, the Shrimp Processing Breakout Group was very certain that feed was processed at temperatures sufficient to inactivate the viruses. Additional research will be necessary to resolve this issue.

2.2.2.3 Colonization Potential

Workshop participants agreed that the potential for viruses to colonize coastal areas is one of the most critical aspects of evaluating the potential for establishment. Workshop participants concluded that there is a high potential for viruses to be associated with many of the pathways identified in this report and a low to high potential that these pathways could lead to introduction of viruses. The breakout groups were certain about association of viruses with these pathways and their entry potential through the pathway; however, they had a high degree of uncertainty about colonization potential. Breakout groups believed that, for most subpathways, there is either a low or medium likelihood that, once introduced, viruses would be able to colonize native shrimp at a local level (i.e., within specific estuaries or embayments). In support of their conclusions, the breakout groups identified the following factors:

- Colonization potential is likely to be related to the magnitude of the source and the frequency of introductions. Therefore, large, frequent sources may have a greater likelihood of colonization than small, intermittent sources.

- Colonization potential is likely to be related to the medium in which the viruses are introduced. For example, viruses introduced within live or dead shrimp are thought to have a greater likelihood of colonization than are viruses introduced via water.

- There is no clear evidence to suggest that colonization has occurred in wild shrimp populations, despite a history of outbreaks in aquaculture operations, the presence of shrimp processing operations, discharges of ballast water, and the use of bait shrimp. (Recent evidence suggests, however, that WSSV-like viruses found in wild shrimp populations in South Carolina coastal waters may not differ from Asian isolates of the virus [Lo et al., in press]).

2.2.2.4 Spread Potential

The breakout groups viewed the potential spread of viruses beyond the initial locus of colonization as an area of uncertainty. During plenary discussion of the reports from the individual breakout groups, workshop participants generally believed that there is a medium probability that viruses could spread beyond the initial locations of colonization.

Breakout groups identified a number of factors significant to evaluating the potential spread of introduced viruses, such as the degree of interaction that would occur among individual wild shrimp and the spatial scale over which shrimp might "mix." Stocks of *P. setiferus* in the southeast Atlantic are thought to be fairly genetically homogeneous, as are the northern and southern populations in the Gulf of Mexico. Workshop participants believed that this suggests the potential for substantial interaction over broad geographic regions, which would promote the spread of viral infection. However, genetic homogeneity may not be the case for other penaeid species. The potential for spread also depends in large part on the time course of the disease, as well as the density of shrimp in wild populations. Breakout group members determined that low shrimp densities are likely to hinder disease spread, whereas high densities are likely to promote transmission. Spread potential is also host dependent and virus specific. It was noted that TSV and IHHNV have low spread potential, and the spread potential of YHV and WSSV is currently unknown. A WSSV-like virus has been found in a variety of crustaceans in southeastern Atlantic waters, but it is unknown at this time if it is the same as the Asian strain of WSSV. (Recent evidence suggests, however, that WSSV-like viruses found in wild shrimp populations in South Carolina coastal waters may not differ from Asian isolates of the virus [Lo et al., in press]). As noted in the JSA report, the presence of other stressors (e.g., low dissolved oxygen and extreme salinity) is also likely to influence the potential for spread of the disease. When WSSV is detected in wild stocks in Asia, it is known to be distributed over wide geographic areas, which suggests that viral disease can spread from an original locus of colonization.

2.2.3 Consequences of Establishment

In continuing to assess the risks to wild populations of shrimp viruses, the breakout groups evaluated the potential ecological effects associated with the establishment of pathogenic shrimp viruses. The breakout groups approached this step of the qualitative risk assessment process by considering the available information on the direct effects of viruses on shrimp. Breakout groups also examined possibly analogous situations based on experience with other diseases and invertebrates. Breakout groups discussed possible effects on ecological structure and function but, due to the limited time available, gave primary attention to direct effects on wild shrimp populations. In the absence of documented information or firsthand knowledge, experts relied primarily on professional judgment to evaluate the consequences of establishment. The breakout groups concluded that there is a high degree of uncertainty in assessing the consequences of establishment.

2.2.3.1 Direct Consequences to Shrimp Populations

In considering the possible consequences of shrimp viruses to shrimp populations at the local level and at the scale of the entire populations or stock, breakout groups evaluated three types of effects:

- Mortality of the infected animal
- Reduction in reproductive rates
- Alteration of the genetic structure of the population

<u>Mortality Effects.</u> Breakout group experts concluded that the direct consequences of the establishment range from low to medium and that effects on the mortality of shrimp are more likely to occur at the local level than at the scale of the entire population or stock. The breakout groups determined that the probability of colonization at a local level is greater than the probability that viruses would spread beyond the local level to a regional population. It is thought that WSSV and YHV are more likely than IHHNV or TSV to cause acute mortality but that IHHNV and TSV are more likely to become endemic.

<u>Reproductive Effects</u>. Breakout group experts focused primarily on factors that would affect reproductive output or recruitment. Experts were aware of no information describing adverse viral effects on the reproductive potential of infected individuals (indicating a potentially important data gap). One expert noted that reproductive output of infected *P. vannamei* brood stock appears to be unaffected by viral infection (F. Jaenike, personal communication). However, in contrast to the previous statement, individual growth impairment in offspring of *P. vannamei* infected with IHHNV has been documented (Fulks & Main, 1992). Assuming that fecundity of female *Penaeus* is an increasing function of size (a phenomenon common in other invertebrate species), workshop participants considered that stunted growth of offspring could result in reduced reproductive output of the second generation. Individual growth impacts could therefore cause population-level effects, although an analysis of any changes in reproduction on shrimp population dynamics would be required to support this conclusion. Workshop participants noted that epidemiologic models show that, in “r-selected” species, effects on reproduction can have greater effects on population size than mortality effects. (Penaeid shrimp can be characterized as “r-selected” organisms because they display an annual life history pattern with high reproductive output and high mortality during early life stages.)

<u>Effects on Genetic Structure and Fitness</u>. Breakout group participants discussed the potential effects of virus colonization on the genetic structure and fitness of wild shrimp populations. One breakout group thought that rapid reductions in population abundance resulting from viral disease could have unknown but potentially important effects on genetic structure by limiting genetic variability (the “founder effect”). One participant cited evidence from Thailand indicating that shrimp populations in the south of Thailand are much less genetically diverse than those from the northern part of the country. It has been hypothesized that this is due to the release of shrimp from aquaculture into the wild. One breakout group discussed whether genetic resistance to viruses differs among populations. Further knowledge of genetic variability among Gulf Coast

shrimp is necessary to make accurate predictions about which area has the highest potential for an epizootic.

Other Information. Other information or lines of evidence that affected the experts' professional judgments about the potential consequences of establishment are in the following:

- Penaeid shrimp can be characterized as "r-selected" organisms because they display an annual life history pattern with high reproductive output and high mortality during early life stages. Thus, penaeid shrimp populations that suffer population reductions in one year can exhibit rapid recovery, and this may reduce the long-term consequences of short-term impacts. In reviewing available information, the breakout group concluded that mass mortalities of adult shrimp may have relatively short-term impacts on standing shrimp stocks. For example, some natural stressors on shrimp (e.g., cold temperatures or freshwater flooding) are known to cause short-term reductions in populations at the local level. Because of high fecundity and migratory behavior, *P. setiferus* is capable of rebounding from a very low population size in one year to a large number in the next, if environmental conditions are favorable. This has been observed off the South Carolina coast several times in the past 50 years (Linder & Anderson, 1956; McKenzie, 1981). In another case, an increase in reproductive output of the Honduran population of *P. vannamei* was reported during a 1994 TSV outbreak. This provides anecdotal support for the concept that demographic compensatory responses may occur in disease-depleted populations, although it was noted that the population changes could have been caused by other factors (Laramore, 1997).

- Along with anecdotal information about the possible long-term effects of viral infections in Latin American and Asian shrimp populations, observations by some workshop participants suggest that direct mortality effects would be relatively transitory. Also, it was suggested that initial outbreaks could lead to enhanced resistance to future viral infection, based on the observation that resistance to IHHNV appears to have increased in all populations tested since the identification of this virus in Hawaiian stocks.

  It should be noted, however, that some workshop participants were concerned that the ability of viral pathogens to persist at low levels in a population could result in long-term adverse population effects. For example, purported virus-induced declines in the population abundances of *P. stylirostris* in the Gulf of California began in 1987 and lasted 6 to 7 years, with stocks now reported to have returned to preoutbreak levels. (The role of IHHNV as the cause of the initial population decline has been the subject of much debate, however.)

- Based on observations from aquaculture situations, it appears that local colonization of shrimp viruses could result in local mortalities of shrimp. For example, TSV and others viruses are known to cause mass mortality on shrimp farms. Experiments with these viruses have documented mortality rates of up to 100 percent. In South Carolina, survival on commercial farms affected by TSV dropped from 63 percent in 1995 (the year prior to the TSV outbreak) to 19 percent in 1996 (the year of the TSV outbreak) (P. Sandifer, personal communication).

- Lines of evidence from other crustacean species indicate an association between an introduced biological agent and subsequent environmental impacts. For example, a crayfish species introduced from California to Europe may likely have served as a carrier to spread the freshwater crayfish plague throughout Scandinavia (Unestam & Weiss, 1970). Unlike short-term natural stressors (e.g., changes in temperature or salinity), an introduced disease organism (biological stressor) is likely to persist in the population.

- No empirical data exist to indicate that historical releases of shrimp virus to the Gulf of Mexico or to southeast Atlantic coastal waters have resulted in population-level impacts. However, no well-designed studies have been conducted to examine the epidemiological conditions within these waters.

2.2.3.2 Effects on Ecological Structure and Function

Workshop participants observed that the introduction of shrimp viruses could affect ecological conditions apart from any direct effects on shrimp; however, experts made only a limited attempt to characterize these consequences, primarily because of a lack of information and because these indirect effects were not a focus of this workshop. Despite these limitations, some of the discussion related to this topic may be helpful to risk managers.

The Aquaculture Breakout Group discussed instances in which other invertebrate species have experienced severe disease consequences. Participants viewed these examples as relevant to the effects of pathogenic viruses on shrimp:

- The near decimation of oysters (*Crassostrea virginica*) by the protozoan pathogens *Haplosporidium nelsoni* and *Perkinsus marinus*, called MSX and dermo disease respectively (Haskin & Andrews, 1988; Andrews, 1996; Burreson & Ragone-Calvo, 1996), has resulted in significant changes in the oyster reef habitat throughout Chesapeake Bay and dramatically reduced the rate at which bay water was filtered by feeding bivalves (Kennedy, 1996).

- Insect/virus associations in which high abundances of the host species promote rapid outbreaks of viral disease, followed by dramatic declines in the host, near-disappearance of the virus, and reestablishment of the host (S. Thiem, personal communication).

- The introduction into Scandinavia of North American crayfish that were carriers of the freshwater crayfish plague *Aphanomyces astaci* (Unestam & Weiss, 1970).

Workshop participants felt that these and similar examples could serve as models for extrapolating potential consequences of viral establishment for shrimp populations. These examples may also serve as models for how ecological systems might be affected by viral outbreaks in shrimp. Either application would require careful analysis to identify similarities and differences relative to the shrimp virus situation.

The "Other Pathways" Breakout Group discussed the potential for viruses to affect estuarine ecology by infecting other species of shrimp, such as grass shrimp. Grass shrimp (*Paleomonetes sp.*) are an important part of the estuarine food web. Many species of fish (and penaeid shrimp) rely on this species as an important prey item. Data from Thailand suggest that grass shrimp may be carriers of one or more of these viruses, but data on infectivity rates and effects are lacking. On the other hand, it was noted that observations in South Carolina confirmed the presence of large populations of apparently healthy *Paleomonetes* in tidal areas near TSV-infected shrimp farms.

2.2.4 Risk Characterization

Using the Aquatic Nuisance Species approach (RAM, 1996; Appendix G), workshop participants characterized the risk of viral introductions to wild penaeid shrimp populations by combining the probability of establishment of the virus with that of the presumed ecological consequences (see Section 2.1). Workshop participants assessed risks to local populations, which they generally defined as the population within a single estuary, and they also considered the long-term effects on the entire population of native shrimp in the Gulf of Mexico and southeastern Atlantic coastal waters.

2.2.4.1 Risk to Local Populations

Workshop participants concluded that the probability of establishment of shrimp viruses in a local estuary ranges from low to medium. The probability of establishment depends primarily on the colonization potential of the particular viruses. However, the probability of establishment could become much greater if virus is introduced repeatedly to the estuary over a long period. Workshop participants generally believed that the impact of such an establishment on the local shrimp population would be moderate. They noted that initial kill rates might be high, but the population would likely recover rapidly due to reintroduction of shrimp from other locations. Therefore, workshop participants characterized the overall long-term risk of virus introductions to the shrimp populations in a local estuary as moderate. (The possibility of longer-term effects is suggested in discussed in Section 2.2.3.1).

Workshop participants also considered the risks posed by shrimp viruses to other components of the estuarine ecosystem to be moderate, although uncertainty surrounding this risk estimate is very high. Of particular concern to participants was coinfection of important food-web species, such as grass shrimp and crayfish. Because both penaeid shrimp and grass shrimp are important food sources for many other estuarine organisms, participants noted that the loss of this food base could have significant effects on other species. Following an initial viral kill of shrimp, fish or wildlife populations that depend on shrimp and other crustaceans as prey sources may take longer to recover than shrimp populations.

Participants raised concerns about the lack of information on the transmissibility of disease from one estuary to another through migration of diseased or infected shrimp. Participants thought that survivors of a local epizootic could move out to sea to reproduce, possibly infect other shrimp and offspring, and then move into adjacent or nearby estuaries. Such an event would expand what appears to be a localized risk into large-scale risk; however, each breakout group that evaluated the potential for spread by natural processes rated the probability of this occurrence as low. Therefore, the risk of a local infection having large-scale consequences is characterized as moderate.

2.2.4.2 Large-Scale Risk

Workshop participants characterized the risk from viral introductions to the entire population of native shrimp along the southeastern Atlantic coast and within the Gulf of Mexico using the same analysis of the establishment pathways combined with that of the potential consequences of establishment on a large geographic scale. Workshop participants concluded that the consequences of virus introduction to the population as a whole would be relatively insignificant, and they characterized the risk as low.

Some participants expressed concern that the genetic structure of the population might be altered and, if viral resistance were linked with appropriate genes, overall fitness of the shrimp could be lowered. One participant noted that alterations to the genetic structure of the population could make the shrimp more susceptible to future infections and to simultaneous environmental stressors, such as weather changes or reduced estuarine salinity, thereby potentially increasing the risk potential. Furthermore, some participants stressed that uncertainty about the long-term ecological consequences of viral introduction will remain high until the effects of virus infection on reproduction can be determined.

2.2.4.3 Summary

Overall conclusions by workshop participants concerning the risks posed by nonindigenous shrimp viruses may be summarized as follows:

- Based on information currently available, most workshop participants believed that the risk to native shrimp from introduction of nonindigenous viruses is low to moderate, although uncertainty is high.

- Most participants agreed that local effects should be given a higher risk ranking than large-scale effects because local effects are more likely to occur.

- Participants suggested that the large amount of uncertainty associated with this risk characterization could be reduced through appropriate laboratory and field studies. The lack of evidence of conclusive viral impacts on worldwide shrimp populations does not derive from published systematic studies but rather is anecdotal. Furthermore, by analogy, other marine invertebrates have experienced severe local impacts from exposure to pathogens (as has been noted in oyster populations in Chesapeake Bay). Also, viruses that have become established in terrestrial insect populations can cause cyclic epizootics and population crashes. Therefore, participants concluded that there is an urgent need to continue efforts to gather available data on shrimp virus effects and to conduct a systematic research effort that could be used to reduce the uncertainty of any subsequent risk assessments.

2.3 RISK MANAGEMENT RELEVANCE

Although this report does not recommend risk management actions, it contains information that may help risk managers with their decisions by:

- Providing insight into the pathways by which shrimp viruses could potentially enter and become established in the marine environment
- Identifying potential consequences to wild shrimp populations at local and stock levels
- Suggesting specific actions and studies that can reduce the uncertainties associated with evaluating the potential risks of shrimp viruses on wild shrimp populations

The ability to make quantitative estimates of the risks of viruses to wild populations of penaeid shrimp is constrained by the amount and type of information that is currently available. The majority of workshop participants believed that it is unlikely that the information required to make quantitative estimates of risk will be available within the foreseeable future. At present, qualitative evaluations can be made.

The ability of workshop participants to address broader ecological risks in a comprehensive manner was limited by available information, but participants thought that this is an important issue that merits further consideration. Furthermore, while the risks that shrimp viruses pose to shrimp aquaculture operations was not part of the scope of the workshop, workshop participants agreed that these risks should be given special attention as part of another technical or management workshop.

3. ACTIONS FOR REDUCING UNCERTAINTY

The qualitative risk assessment conducted during the workshop revealed several critical sources of uncertainty. Further improvement in the ability to estimate risks to wild populations of shrimp will require reducing uncertainty in these key areas.

Workshop participants discussed the relative importance of actions for reducing uncertainty. Some participants stressed that, to reduce uncertainty, risk management actions need to occur in parallel with research, monitoring, and other actions.

Most workshop participants generally believed that particular emphasis should be given to the following actions for reducing uncertainty:

- Improved diagnostic methods
- Surveys of wild shrimp populations for the presence of nonindigenous viruses and for genetic composition
- Experiments to reduce uncertainties surrounding virus transmission and virulence
- Field epidemiological studies

3.1 DIAGNOSTIC METHODS

Workshop participants determined that improvements to existing diagnostic methods and development of new diagnostic tools are a very high priority. Several participants noted that without adequate diagnostic methods, other risk assessment elements cannot be well studied or adequately evaluated. Other participants noted that many valuable diagnostic tools currently exist. Several key needs were identified during the workshop:

- There is a significant need to develop new diagnostic procedures. Some molecular probe applications and bioassay tests are available, although several workshop participants noted that the sensitivity of existing bioassay tests needs to be improved. One participant also cited the need to develop cell culture tests for crustacea, noting that new technologies are available to assist in developing cell cultures, but money and lack of equipment have been major obstacles.

- Tests for infectivity are needed to establish the threshold number of viruses that would be required for colonization potential. At least two tests should be employed, such as a PCR and ELISA or a PCR and a bioassay.

- Current diagnostic applications are focused on detecting viruses in the animal itself. Although some preliminary efforts have been made to detect viruses in environmental media (e.g., to identify the presence of WSSV using water concentration techniques and PCR), techniques to detect viruses in effluent streams, sediment, and other environmental media need to be improved.

- There appears to be considerable variability among laboratories in the procedures for using available diagnostic tools. Procedures for using diagnostic tools should be standardized so that both the credibility and limitations of diagnostic tools can be established.

3.2 SURVEYS OF WILD SHRIMP POPULATIONS

Participants identified the need to survey native shrimp populations to develop baseline information on viruses in wild stocks. It was noted that some monitoring activity has been conducted in the coastal waters of South Carolina and Texas. Participants generally believed that it was important to proceed with field surveys despite the current limitations of diagnostic methods. Participants suggested that because of these limitations, current survey efforts should include the archiving of samples to be evaluated pending development of improved diagnostics.

Workshop participants noted that monitoring surveys should include genetic characterization of wild populations. To date, only limited studies have been conducted. (In one study that is underway, molecular techniques are being used to determine the degree of genetic variability between populations of *P. setiferus* in the Gulf of Mexico and the U.S. southeastern Atlantic coastal region.) Participants suggested that surveys should be focused both in areas that may have experienced the release of nonindigenous viruses and areas where it is unlikely that prior release has occurred.

3.3 EPIDEMIOLOGY OF SHRIMP VIRUS TRANSMISSION

Workshop participants identified a need for well-designed experiments to improve understanding of the pathogenicity of viruses in native shrimp. In particular, studies are needed on virulence, distribution in various shrimp tissues, and rates of transmission, susceptibility, and recovery. Some suggested that laboratory experiments would be hindered by inadequacies in current techniques to identify pathogens and by the absence of diagnostic methods specific to identifying viruses in various environmental media. Given existing techniques for quantifying the amount of virus present, participants noted that currently it is most feasible to conduct qualitative transmission studies in which the amount of virus is estimated on a relative basis.

In other discussions, participants identified the need to understand not only mortality effects but also the consequences of infection on shrimp reproduction and growth. It is recognized that there are significant differences in viral pathogenesis among the four different viruses and the relative ability of the viruses to affect mortality, growth, and reproduction.

Participants also identified the need to develop a better understanding of the transmission of viruses from one species to another (i.e, between penaeid species and between penaeid and nonpenaeid species).

One participant stated that the most important reason to improve understanding of the epidemiology of shrimp viruses is to help identify mitigation measures (e.g., for aquaculture as a pathway).

3.4 FIELD EPIDEMIOLOGICAL STUDIES

In addition to laboratory-based experiments, most participants believed that a parallel effort involving field epidemiology could yield information helpful for understanding the prevalence and potential effects of viruses in wild shrimp populations. Field epidemiological studies may not provide the same level of understanding of detailed mechanisms as would laboratory experiments.

Participants suggested that field epidemiological studies could make use of existing information from Latin America and Southeast Asia. Information would be sought on:

- The extent to which native shrimp populations in these areas may have been exposed to viruses
- The presence of viruses within these populations
- The observed effects (or lack thereof) of viruses on shrimp abundance and recruitment
- Possible ecological effects

Others suggested that the known locations of shrimp virus prevalence around the world should be documented and mapped so that potential sources can be identified.

3.5 LOWER PRIORITY RISK-RELEVANT RESEARCH AREAS

Workshop participants identified other areas, in addition to the four priority areas listed previously, where additional research is needed to improve the ability to estimate risks to wild shrimp populations.

3.5.1 Viral Persistence

Some participants noted the need to develop better techniques and to conduct experiments to evaluate the persistence of viruses in effluent streams, sediment, and other environmental media. It was noted that experiments should couple viral persistence with viral infectivity. For example, participants noted that IHHNV can be detected in sediments for 24 days; however, the duration of infectivity is unknown.

3.5.2 Compensatory Mechanisms

Participants felt that it is important to develop a better understanding of the compensatory mechanisms of native shrimp species in response to viral disease outbreaks. Research is needed to:

- Understand genetics and disease resistance (i.e., the need to improve understanding of the relationship between population genetics and the identification of disease-resistant phenotypes and how particular phenotypes develop resistance to a particular virus).

- Determine whether shrimp populations compensate for increased mortality with increased reproduction.

- Compile information on the shrimp immune-like response to viral infection. It was noted that coupling our understanding of target-organ sensitivity with information about resistance will improve the ability to predict which shrimp are likely to become carriers.

3.5.3 Monitoring of Imported Shrimp

Participants identified the need to monitor virus levels in imported shrimp using tests such as PCR and bioassay. Some suggested that, in terms of risk reduction, monitoring imported shrimp should be a higher priority than monitoring wild shrimp populations because of the high volume of imported shrimp.

3.5.4 Development of Suitable Population Models

Suitable population models are needed to evaluate the consequences of various virus-induced mortality or reproductive impairment scenarios. Because of the commercial importance of shrimp, workshop participants believed that it is highly likely that population models exist for these species. Additionally, a large body of catch statistics could be subject to time series analysis in concert with known periods of virus outbreaks or other environmental stressors, such as storm events. These types of data may be available for foreign fisheries as well. By using population models, constants for infection and transmission rates, and transport and fate, a modeling framework could be created to examine specific hypotheses. Sensitivity analyses could then be performed to determine which parameters are most important and contribute the most uncertainty. Research could then be directed to reduce uncertainty.

3.5.5 Other Risk-Related Research Needs

Other risk-related research needs identified by workshop participants include:

- Procedures for disinfection and eradication of large-scale outbreaks in aquaculture settings
- Genetic and biochemical characterizations of the viruses
- Research to improve understanding of factors that exacerbate expression of viral disease under conditions of high densities and high nutrients found in aquaculture settings
- Targeted surveys of nonpenaeid species (e.g., grass shrimp, crayfish, and micro-crustacea) to determine if they are susceptible to, or carriers of, nonindigenous viruses

4. SUMMARY

This section provides a brief summary of the results of the workshop. Topics include the qualitative risk assessment process; the need for a future, more comprehensive risk assessment; risk-relevant research needs; and areas of additional concern.

4.1. QUALITATIVE RISK ASSESSMENT PROCESS

Workshop participants conducted a qualitative assessment of risks by considering the:

- Likelihood of viruses being present in the pathway
- Ability of the viruses to survive transit in the pathway
- Colonization potential of the viruses (in native shrimp)
- Spread potential of the virus within native shrimp populations
- Consequences of establishment

In general, workshop participants believed that viruses could be in pathways leading to coastal environments and that they could survive in these pathways. Participants concluded that there is some potential for viruses to colonize native shrimp in a localized area, such as an estuary or an embayment, near the point of entry into the marine system. Participants had widely divergent views on the potential for viruses to spread beyond the initial local area of colonization, and this divergence reflected the large uncertainty associated with this aspect of exposure. Participants considered the potential for localized colonization and subsequent spread to be a critical aspect of evaluating the potential establishment of viruses in native shrimp.

Participants considered the consequences of virus establishment at a local level (e.g., within an individual estuary) as well as within the offshore stocks. Workshop participants discussed the impact of such an establishment on the local shrimp population. Initial kill rates might be high, but the population would be likely to recover rapidly due to reintroduction of shrimp from other locales. The risk from viral introductions to the entire population of native shrimp along the southeastern Atlantic coast and within the Gulf of Mexico was thought to be relatively insignificant, and workshop participants characterized this risk as low. Concern was expressed that certain effects (e.g., effects on genetic structure of shrimp and on the ecological system) may be difficult to assess.

4.2. COMPREHENSIVE RISK ASSESSMENT NEEDS

Most workshop participants concluded that, given the current knowledge base, it is infeasible to conduct a more comprehensive, quantitative estimate of risk. Most participants believed that, at present, qualitative evaluations can be made, but these are accompanied by large uncertainties. Participants agreed that there is a need to continue efforts to gather available data on shrimp virus effects and to conduct a systematic research effort that could be used to reduce the uncertainty in any subsequent risk assessments.

4.3. RESEARCH NEEDS

Workshop participants identified a number of areas in which further research and information would improve the assessment of risks and the evaluation of current conditions, with particular emphasis on the following areas:

- **The improvement of existing and the development of new diagnostic methods for viruses in shrimp and environmental media.** These methods are essential for all research studies and monitoring programs and for determining if viruses are present in imported shrimp, cultures used for aquaculture, and other possible pathways.

- **Surveys of wild shrimp populations.** Baseline information on the presence of viruses in native shrimp populations would provide insight into the extent to which populations already carry viruses. Baseline information would also be useful for supporting epidemiological studies. Baseline studies could proceed even though there are limitations with current diagnostic methods. Well-designed studies would be enhanced by including an examination of the genetic structure of the populations.

- **Epidemiology of shrimp virus transmission.** Workshop participants identified a need for well-designed experiments to improve understanding of the pathogenicity of viruses in native shrimp.

- **Field epidemiological studies.** In addition to laboratory-based experiments, participants believed that a parallel effort involving field epidemiology could yield information helpful for understanding the prevalence and potential effects of viruses in wild shrimp populations.

4.4. ADDITIONAL AREAS OF CONCERN

Workshop participants identified the following areas of concern, in which additional efforts should be focused:

- **Management implications of shrimp viruses.** It was recommended that a risk management workshop be held, focusing on impacts to natural resources and on possible impacts on shrimp importation, processing, and aquaculture operations.

- **Risks of shrimp viruses to aquaculture operations.** Workshop participants also recommended that a separate workshop be held on this topic.

- **Risks of shrimp viruses to nonpenaeid species.** Because this workshop was limited to evaluating the direct effects of viruses on wild shrimp populations, participants recommended that additional effort be directed toward evaluating nonpenaeid shrimp species (e.g., grass shrimp) and other species that could be impacted by the viruses (e.g., crabs, amphipods, and copepods).

5. REFERENCES

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